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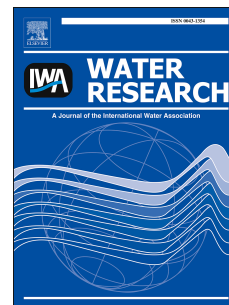
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# Characterisation of dissolved organic matter fluorescence properties by PARAFAC analysis and thermal quenching

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## Abstract

The fluorescence intensity of dissolved organic matter (DOM) in aqueous samples is known to be highly influenced by temperature. Although several studies have demonstrated the effect of thermal quenching on the fluorescence of DOM, no research has been undertaken to assess the effects of temperature by combining fluorescence excitation – emission matrices (EEM) and parallel factor analysis (PARAFAC) modelling. This study further extends previous research on thermal quenching by evaluating the impact of temperature on

the fluorescence of DOM from a wide range of environmental samples, in the range 20° C - 0° C. Fluorescence intensity increased linearly with respect to temperature decrease at all temperatures down to 0° C. Results showed that temperature affected the PARAFAC components associated with humic-like and tryptophan-like components of DOM differently, depending on the water type. The terrestrial humic-like components, C1 and C2 presented the highest thermal quenching in rural water samples and the lowest in urban water samples, while C3, the tryptophan-like component, and C4, a reprocessed humic-like component, showed opposite results. These results were attributed to the availability and abundance of the components or to the degree of exposure to the heat source. The variable thermal quenching of the humic-like components also indicated that although the PARAFAC model generated the same components across sites, the DOM composition of each component differed between them. This study has shown that thermal quenching can provide additional information on the characteristics and composition of DOM and highlighted the importance of correcting fluorescence data collected *in situ*.

**Keywords:** fluorescence spectroscopy; thermal quenching; dissolved organic matter; parallel factor analysis; temperature correction

53

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57

58       **1. Introduction**

59       In recent years, fluorescence spectroscopy has been  
60       increasingly applied to the analysis of aqueous dissolved  
61       organic matter (DOM). The effectiveness of this technique in  
62       water quality analysis has been proven by studies on numerous  
63       types of water systems (Drozdowska, 2007; Kelton et al., 2007;  
64       Murphy et al., 2008; Ghervase et al., 2012; Kothawala et al.,  
65       2012; Carstea et al., 2014). Fluorescence has been correlated  
66       with standard parameters such as biological oxygen demand  
67       (Reynolds and Ahmad, 1997; Hudson et al., 2008; Hur and  
68       Kong, 2008), total organic carbon (Vodacek et al., 1995),  
69       nitrogen and chemical oxygen demand (Hur and Cho, 2012;  
70       Bridgeman et al., 2013). Due to its potential, researchers have  
71       applied fluorescence spectroscopy in studies such as the  
72       monitoring of riverine DOM and diesel pollution (Spencer et  
73       al., 2007; Carstea et al., 2010), analysis of recycled waters  
74       (Henderson et al., 2009), evaluation of drinking water treatment  
75       processes (Bieroza et al., 2009; Shutova et al., 2014),  
76       monitoring of viral abundance in wastewater (Pollard, 2012),  
77       quantification of pesticides (Ferretto et al., in press) or testing  
78       of potable waters microbial quality (Cumberland et al., 2012).

79 The intensive use of fluorescence spectroscopy in water quality  
80 analyses arises from its advantages, which include high  
81 sensitivity, small quantities of sample needed, very little or no  
82 sample preparation and short measuring time (Coble, 1996;  
83 Birdwell and Valsargis, 2010). However, the fluorescence  
84 signal can be affected by so-called “matrix effects” which  
85 include inner filter effects and fluorescence quenching  
86 (Lakowicz, 2006; Henderson et al., 2009; Korak et al., 2014).  
87 With regard to fluorescence quenching, it has been shown that  
88 fluorescence spectroscopy is highly sensitive to temperature  
89 variations. An increase in temperature increases the probability  
90 of the excited electrons returning to ground state through  
91 radiationless decay. Baker (2005) studied temperature  
92 quenching on several types of water samples and observed a  
93 decrease in fluorescence intensity ranging from 16 % to 48 %,  
94 depending on the samples and DOM component analysed.  
95 Elliott et al. (2006) observed a decrease in fluorescence of more  
96 than 40 % for fluorophores produced by bacterial cultures  
97 isolated from river samples and Seredynska-Sobecka et al.  
98 (2007) studied thermal quenching on colloids obtaining similar  
99 results. However, in each case the researchers did not study the  
100 impact of temperature on DOM fluorescence below 10° C, due  
101 to condensation which could form on the cuvette walls.  
102 Patsayeva et al. (2004) and, more recently, Watras et al. (2011)  
103 have analysed thermal quenching to almost 5° C and developed

104 a correction method for fluorescence spectra but both research  
105 teams concentrated only on marine water samples.  
106 Consequently, no research has been made, so far, to study  
107 fluorescence thermal quenching below 5° C on water samples  
108 from a wide range of different sources.

109 This study seeks to characterise the fluorescence  
110 properties of DOM, from water samples with different sources,  
111 using thermal quenching and the combination of excitation –  
112 emission matrices (EEM) and parallel factor analysis  
113 (PARAFAC). Several studies have shown that PARAFAC is a  
114 powerful tool in separating and analysing DOM components  
115 (Ohno et al., 2008; Yamashita and Jaffe, 2008; Gueguen et al.,  
116 2011; Meng et al., 2013; Murphy et al., 2014; Sanchez et al.,  
117 2014; Yang et al., 2014). Specifically, the aims of this study  
118 were: (1) to investigate the response of DOM, from different  
119 sources (urban and rural areas), at low temperatures for a better  
120 understanding of DOM characteristics; (2) to evaluate the  
121 impact of temperature on the most labile fractions of DOM; (3)  
122 to assess the potential of applying the Watras et al. (2011)  
123 correction tools at temperatures below 5° C; (4) to investigate  
124 the use of EEM-PARAFAC tool combined with thermal  
125 quenching to improve our understanding of DOM character. To  
126 date, EEM-PARAFAC has not been applied to the investigation  
127 of thermal quenching of DOM components from water samples  
128 and could provide a better understanding of DOM properties.

129

130 **2. Materials and Methods**131 **2.1 Sample preparation and analysis**

132 Samples were collected from two areas: Birmingham and  
 133 Buxton, located in the Midlands area, UK (Fig. 1). The  
 134 sampling sites, with different characteristics, were selected to  
 135 reflect a gradient from rural to urban areas. In Birmingham, 5  
 136 types of water were sampled, hereafter named: brook (Sutton  
 137 Park), lake (Sutton Park), pond (Edgbaston pond), surface  
 138 runoff from storm sewers (University of Birmingham campus)  
 139 and canal (Worcester and Birmingham Canal). Brook and lake  
 140 samples were collected from Sutton Park, which is a National  
 141 Nature Reserve and presents a relatively rural, pristine  
 142 character (<http://www.birmingham.gov.uk/suttonpark>). Canal,  
 143 storm sewer and pond samples were collected from an urban  
 144 zone; however, the pond was located in a small park with lower  
 145 anthropogenic activity compared to canal and storm sewer.  
 146 From Buxton, a river water sample was collected. Buxton town  
 147 is located along the Wye River, within The Peak District  
 148 National Park, having low anthropogenic impact, according to  
 149 the Environment Agency  
 150 (<http://www.peakdistrict.gov.uk/microsites/sopr/landscape/river>  
 151 -quality).

152 Water was sampled in polypropylene bottles, cleaned with  
 153 10 % HCl and thoroughly rinsed with deionised water prior to



154 use. All measurements were performed within 24h from  
155 collection. The samples were measured for conductivity, pH,  
156 dissolved organic carbon (DOC) and absorbance, from 200 nm  
157 to 700 nm. Conductivity and pH were measured using a Myron  
158 meter, absorbance measurements were made with a WPA  
159 lightwave UV-VIS diode-array S2000 spectrophotometer and  
160 DOC with a Shimadzu TOC-Vcpn analyzer.

161 Fluorescence EEMs were recorded using a Varian Cary  
162 Eclipse spectrofluorometer, with the following parameters:  
163 excitation wavelength domain 200 – 400 nm, emission  
164 wavelength domain 280 – 500 nm, steps of 5 nm and 2 nm for  
165 excitation and emission, respectively, and slits of 5 nm. The  
166 instrument stability was checked by recording the Raman  
167 values (at excitation wavelength 348 nm and emission  
168 wavelength 395 nm) before each set of measurements. The  
169 average Raman value was 24.38 a.u. with a standard deviation  
170 of 0.58. The fluorescence intensity of all spectra were  
171 normalized to a maximum value of 1000 a.u. and corrected to  
172 the average Raman value. Every set of measurements was made  
173 in triplicate in order to check the instrument reproducibility ( $\pm$   
174 5%).

175 The temperature was decreased gradually from 20<sup>0</sup> C to  
176 0<sup>0</sup> C, by the use of a Peltier temperature controller, recording  
177 EEMs at every 0.5<sup>0</sup> C. Each set of measurements lasted for 90  
178 min, to ensure gentle cooling of the sample. Below 6<sup>0</sup> C,

179 condensation usually forms on the cuvette outer walls, but, in  
180 this study, it was eliminated by inserting dessicant bags inside  
181 the sample chamber. The reduction in condensation was  
182 checked by recording fluorescence spectra at periodic time  
183 intervals and at the established temperature range. The  
184 conditions with no condensation were obtained when silica gel  
185 bags had been kept in the sample chamber for 26 hrs.  
186 Throughout the experimental period, the dessicant bags were  
187 periodically replaced. All samples were filtered with 0.7  $\mu\text{m}$   
188 Whatman GF/C paper filters prior to cooling and analysis.

## 189 **2.2 PARAFAC analysis**

190 PARAFAC was performed on a set of 697 EEMs  
191 (including triplicates) for varying temperatures for the six water  
192 sources described above. Although only 6 different water  
193 sources have been used in PARAFAC modelling, they provide  
194 a good variation in terms of spectral properties and a large  
195 number of samples helped to avoid any potential  
196 autocorrelation effects during the split-half validation. Prior to  
197 modeling, EEMs were pre-processed in Matlab using custom-  
198 written functions to remove redundant spectral areas ( $\lambda_{\text{ex}} < 220$   
199 nm,  $\lambda_{\text{ex}} > \lambda_{\text{em}}$ ,  $2 \lambda_{\text{ex}} < \lambda_{\text{em}}$ , Raman and Rayleigh scatter)  
200 (Bieroza et al., 2011). Pre-processed EEMs were normalized to  
201 the Raman scatter peak of water using procedure described in  
202 Lawaetz and Stedmon (2009). The PARAFAC model was  
203 fitted and validated using the DOMFluor toolbox for Matlab

(Stedmon and Bro, 2008). The final four-component model was chosen based on the percentage of variance explained, core-consistency diagnostic (Bro and Kiers, 2003), the results of the split-half analysis and visual inspection of the excitation and emission loadings (Table 1).

### 3. Results and Discussion

#### 3.1 Fluorescence properties of DOM

The four fluorescence components identified in the water samples are shown in Fig. 2. Component 1 ( $\lambda_{\text{ex}} \sim 225$  nm and  $\sim 330$  nm,  $\lambda_{\text{em}} \sim 460$  nm) is associated with terrestrial humic substances, being similar to the PARAFAC components found by Stedmon and Markager (2005), Murphy et al. (2008; 2011; 2014), Kowalczyk et al. (2009), Williams et al. (2010), Baghth et al. (2011), Yamashita et al. (2011), Ishii and Boyer (2012), Kothawala et al. (2012), Maie et al. (2012) and Yamashita et al. (2013). These studies have shown that this component is ubiquitous in water systems, having a primary terrestrial source and a secondary microbial source of DOM. In addition, C1 is dominated by biological production and is partially degraded. According to Fellman et al. (2010) and Ishii and Boyer (2012), C1 has high molecular weight ( $>1000$  Da) and presents a high degree of hydrophobicity and aromaticity.

Component 2 (C2), found at  $\lambda_{\text{ex}} \sim 225$  nm and  $\sim 330$  nm,  $\lambda_{\text{em}} \sim 410$  nm, belongs to the group of humic fluorophores,

229 based on the studies of Stedmon and Markager (2005), Murphy  
230 et al. (2008; 2014), Williams et al. (2010), Yamashita et al.  
231 (2011), Ishii and Boyer (2012), Maie et al. (2012). These  
232 studies show that C2 is found mostly in DOM dominated by  
233 terrestrial sources and is photochemically produced. C2  
234 presents minimal biodegradation and, according to Ohno et al.  
235 (2010), has low molecular weight (<665 Da).

236 The third component, C3,  $\lambda_{\text{ex}}$  ~225 and ~275 nm,  $\lambda_{\text{em}}$   
237 ~350 nm, indicated the presence of a tryptophan-like fraction,  
238 in accordance with the results of Stedmon and Markager  
239 (2005), Williams et al. (2010), Murphy et al. (2011; 2014),  
240 Maie et al. (2012), Yamashita et al. (2013), Shutova et al.  
241 (2014). Furthermore, Fellman et al. (2010) and Kothawala et al.  
242 (2012) found that this component is a product of  
243 autochthonous, microbial processing.

244 Component 4 (C4) ( $\lambda_{\text{ex}}$  ~240 and ~320 nm,  $\lambda_{\text{em}}$  ~380 nm)  
245 is linked to the humic substances, as shown by Stedmon and  
246 Markager (2005), Murphy et al. (2008; 2011; 2014), Graeber et  
247 al. (2012), Kothawala et al. (2012), Maie et al. (2012), Ishii and  
248 Boyer (2012) and Yamashita et al. (2013). These studies  
249 demonstrate that C4 indicates recent biological production and  
250 is often defined as a microbial humic-like component (Murphy  
251 et al., 2011; Maie et al., 2012; Yamashita et al., 2013). Ishii and  
252 Boyer (2012) report that C4 has an intermediate molecular  
253 weight, between C1 and C2.

254           The mean fluorescence values of component scores and  
255   the relative abundance of each component to the total  
256   fluorescence intensity are presented in Table 2. C1 and C2 are  
257   most abundant at the brook and lake samples, followed by the  
258   river and pond samples and are the least abundant at the canal  
259   and storm sewer samples. The abundance of C3 and C4 is  
260   higher at the canal and storm sewer samples compared to the  
261   other samples. A correlation between C1 and C2 was observed  
262   ( $r_s = 1.00$ ,  $n = 7$ ,  $p < 0.001$ ), which indicated that all samples  
263   contained both high and low molecular weight DOM  
264   compounds and with hydrophobic and hydrophilic characters,  
265   in almost equal proportions. In addition, a strong correlation  
266   between C3 and C4 was calculated ( $r_s = 0.93$ ,  $n = 7$ ,  $0.01 > p >$   
267    $0.005$ ) showing a close relationship between the tryptophan-  
268   like compound and the microbial humic-like fraction. Despite  
269   the low degrees of freedom for both correlations ( $df = 5$ ), given  
270   by the replication in the dataset, the correlations were  
271   considered significant since the components tendencies were  
272   similar.

273           Based on these results, it was observed that the brook,  
274   lake and river samples, which were collected from relatively  
275   pristine areas, contained DOM with a strong humic-like  
276   character, indicating low anthropogenic contamination. While  
277   canal and storm sewer samples showed a high abundance of  
278   tryptophan, typically associated with microbial material

(Kothawala et al., 2012), indicating the presence of anthropogenic-derived matter (Meng et al., 2013; Carstea et al., 2014). The distinction between urban and rural samples is better reflected by the C3/C1 ratio (Table 2): brook, lake and river samples with a rural character had the lowest values, pond sample had an intermediate urban and rural character due to the sampling location in an urban park, and canal and storm sewer with an urban impact showed the highest C3/C1 values. Canal and storm sewer also presented similar values for DOC and absorbance (Table 3). Furthermore, rural samples showed higher DOC and absorbance values compared to the other samples. The highest conductivity values were detected at the canal and pond samples, while the lowest values were seen at the storm sewer sample. The values for pH were recorded within the range of 6.7 and 8.1.

### 3.2 Thermal quenching of humic-like components

The fluorescence response to temperature variation, between 20<sup>0</sup> C and 0<sup>0</sup> C, for the humic-like components C1, C2 and C4 is shown in Figure 3 (a, c and e). All three components exhibit a linear fluorescence increase with temperature decrease. Similar linearity was reported in the studies of Baker et al. (2005), Seredynska-Sobecka et al. (2007) and Watras et al. (2011) on thermal quenching of DOM fluorescence, in the range of 45<sup>0</sup> C - 5<sup>0</sup> C. Although, PARAFAC components

303 showed similar linear trends at all samples, the degree of  
304 temperature impact was highly variable.

305 Figure 3 (b, d, f) presents the slope of fluorescence  
306 intensity decrease per degree Celsius. C1 shows the highest  
307 slope at the rural samples, lake and brook, followed by the  
308 pond and storm sewer samples, while the lowest values have  
309 been seen at the river and canal samples. Similar sample  
310 variability of slope was observed at C2. The last humic-like  
311 component, C4, presents the highest slope at the urban samples,  
312 storm sewer and canal, whilst the lowest have been seen at the  
313 rural samples. It must be noted that although the PARAFAC  
314 model is consistent across all samples, the degree of thermal  
315 quenching is variable between them. This suggests that each  
316 humic-like PARAFAC component is comprised of more than  
317 one fluorophore.

318 Overall, C1 exhibits a higher slope of fluorescence  
319 intensity decrease compared to C2 and C4, indicating that this  
320 component might be more environmentally impacted.  
321 Seredynska-Sobecka et al. (2007) reported that the humic-like  
322 fraction has high sensitivity to thermal quenching, especially at  
323 the small size fractions ( $< 0.1 \mu\text{m}$ ). Furthermore, Ohno et al.  
324 (2008), Yamashita and Jaffe (2008) and Mounier (2010)  
325 proved, by studying the interaction between DOM and metal  
326 ions, that this component was more likely to suffer fluorescence  
327 quenching, compared to the other humic-like components. This

328 indicated that C1 is more sensitive to environmental changes  
329 relative to C2 and C4. Moreover, C2 and C4, which are  
330 resistant to further degradation, after photochemical and  
331 biological production and degradation (Ishii and Boyer, 2012),  
332 are probably less affected by temperature changes. The high  
333 slope of C1 could also be associated with the relative  
334 abundance of fluorescence intensity, as higher slope has been  
335 observed at samples with high abundance. Hence, C1 could be  
336 more readily available for thermal quenching compared to C2  
337 and C4.

### 338 **3.3 Tryptophan-like component behaviour to** 339 **temperature changes**

340 Tryptophan-like component, C3, shows the same linearity  
341 as the humic-like components (Fig. 4a), in accordance with the  
342 results of Baker (2005) and Elliott et al. (2006). Furthermore,  
343 variable gradients of fluorescence decrease per degree Celsius  
344 (slope) have been observed (Fig. 4b). The highest slope has  
345 been seen at the storm sewer and canal samples, followed by  
346 the lake, pond and river samples, while the lowest has been  
347 observed at the brook sample.

348 In contrast to the humic-like components, C3 slope could  
349 be associated to a lesser extent with the relative abundance of  
350 fluorescence intensity (Table 2). Although, C3 is more  
351 abundant in the canal sample, compared to storm sewer, it  
352 shows a lower slope value. According to Baker (2005) the



353 degree of thermal quenching relates to the exposure of the  
354 fluorophore to the heat source. These findings suggest that C3,  
355 belonging to storm sewer DOM, contains more exposed  
356 tryptophan compared to the canal sample. The same  
357 assumption could apply to the lake sample C3, which presents a  
358 high slope value, despite the low abundance relative to river  
359 and pond samples. The results suggest that free tryptophan  
360 could be a dominant component in storm sewer and lake  
361 samples and is, therefore, more easily quenched with increasing  
362 temperature.

363       The various responses of PARAFAC components scores  
364 to temperature fluctuations can have a large impact on *in situ*  
365 fluorescence measurements, especially when comparing  
366 experiments from several locations made in different seasons or  
367 times of the day. Consequently, the fluorescence spectra need  
368 to be corrected for temperature before comparison studies can  
369 be made. The temperature correction tool, developed by Watras  
370 et al. (2011), uses a temperature coefficient, which is the ratio  
371 between the slope of the fluorescence intensity as a function of  
372 temperature change, from 20<sup>0</sup> C to 5<sup>0</sup> C and the intercept, at the  
373 reference temperature of 20<sup>0</sup> C. However, their studies have  
374 been performed on lake water and could not account for  
375 variations between different types of water samples. The slope,  
376 calculated in the present study, shows the same linear trend of  
377 increase below 5<sup>0</sup> C, indicating that the temperature correction

378 tool developed by Watras et al. (2011) can be applied even to  
379 fluorescence spectra of samples measured below 5<sup>0</sup> C.

380

#### 381 4. Conclusions

382 This study presents the first investigation of DOM  
383 fluorescence properties, at low temperatures, with EEM-  
384 PARAFAC. The impact of temperature on the individual  
385 PARAFAC components in DOM, from several water samples,  
386 was evaluated by decreasing the temperature from 20<sup>0</sup> C to 0<sup>0</sup>  
387 C. This analysis extends the fluorescence thermal quenching  
388 studies, made by other researchers, in the range of 45<sup>0</sup> C – 5<sup>0</sup> C.  
389 Results have shown that fluorescence intensity has a linear  
390 increase, as temperature decreased from 20<sup>0</sup> C to 0<sup>0</sup> C. Thus,  
391 the temperature correction tools developed by Watras et al.  
392 (2011) can be applied to fluorescence spectra of samples  
393 measured at temperatures below 5<sup>0</sup> C.

394 It has been found that temperature affects the PARAFAC  
395 components associated with the tryptophan-like and humic-like  
396 fractions differently, depending on DOM character of each  
397 sample. The humic-like components, C1 and C2 present the  
398 highest thermal quenching at the rural samples and the lowest  
399 at the urban samples, while C4 show opposite results. The data  
400 indicate that, while the PARAFAC model is consistent across  
401 all samples, the degree of thermal quenching varies between  
402 them, suggesting that each humic-like PARAFAC component

403 is comprised of more than one fluorophore. Furthermore,  
404 thermal quenching has shown that, among the humic-like  
405 components, C1 is more environmentally impacted but, at the  
406 same time, more readily available to quenching compared to C2  
407 and C4. The tryptophan-like component presents the highest  
408 slope of fluorescence decrease per degree Celsius in the urban  
409 samples and the lowest at the rural samples. Thermal quenching  
410 has evidenced that free tryptophan residues, from the  
411 tryptophan-like fraction, are dominant at the storm sewer and  
412 lake samples, due to the direct exposure of the fluorophore to  
413 the heat source.

414       Considering that a growing body of literature stresses the  
415 importance of using fluorescence for *in situ* measurements, the  
416 analysis of temperature effects on DOM is highly important, as  
417 the fluorescence signal of each DOM component is variably  
418 quenched depending on temperature. Therefore, we recommend  
419 correction of the fluorescence spectra recorded at temperatures  
420 below 20<sup>0</sup> C. However, it is necessary to be aware of the  
421 potential multi-fluorophoric nature of the PARAFAC humic-  
422 like components, which may lead to variable results between  
423 sites.

424

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629 Figure captions

630 Fig. 1 Map with the sampling points from Birmingham and  
631 Buxton (Map of UK adapted from © OpenStreetMap  
632 contributors, CC BY-SA, Open Database License 2010).

633 Fig. 2. Excitation and emission matrices of the four PARAFAC  
634 components.

635 Fig. 3 Linear relationship between PARAFAC scores and  
636 temperature, and the slope: (a) and respectively (b) component  
637 1, (c) and (d) component 2, (e) and (f) component 4.

638 Fig. 4 Linear relationship between PARAFAC scores and  
639 temperature (a) and the slope (b) for component 3.

Table 1. A summary of the PARAFAC models fitted to fluorescence dataset with the following constraints: sample mode – non-negativity, excitation and emission modes – non-negativity and unimodality

Number of components	Convergence (Yes, No)	Sum of squares of errors	Total variance explained (%)	Core-consistency (%)	Split-half analysis validation (Yes, No)
1	Yes	27056	96	100	Yes
2	Yes	23183	96	-87	Yes
3	Yes	5540	99	41	Yes
4	Yes	4860	99	6	Yes
5	Yes	4124	99	0	Yes
6	Yes	4021	99	2	Yes
7	Yes	3669	99	1	Yes

Table 2. DOM fluorescence results of the water samples.

Samples	Mean value of component scores (a.u.) (SD*)						Relative abundance of fluorescence intensity (%)**			
	C1	C2	C3	C4	C3/C1	Total	C1	C2	C3	C4
Brook	30.9 (1.4)	20.0 (1.1)	1.7 (0.2)	0.2 (0.1)	0.1	52.8	59	38	3	0
Lake	23.0 (1.0)	16.5 (1.0)	4.3 (0.4)	0.9 (0.1)	0.2	44.7	51	37	10	2
River	9.5 (0.4)	5.7 (0.3)	3.0 (0.3)	1.2 (0.1)	0.3	19.3	49	29	15	6
Pond	14.9 (0.7)	11.7 (0.7)	6.8 (0.4)	6.6 (0.3)	0.5	39.9	37	29	17	16
Storm Sewer	13.6 (0.5)	11.5 (0.6)	15.0 (0.8)	9.7 (0.4)	1.1	49.8	27	23	30	19
Canal	6.6 (0.3)	4.9 (0.3)	9.8 (0.4)	6.4 (0.3)	1.5	27.6	24	18	35	23
Blank	0.1 (0.0)	0.3 (0.0)	0.1 (0.0)	0.0 (0.0)	-	0.6	22	52	19	7

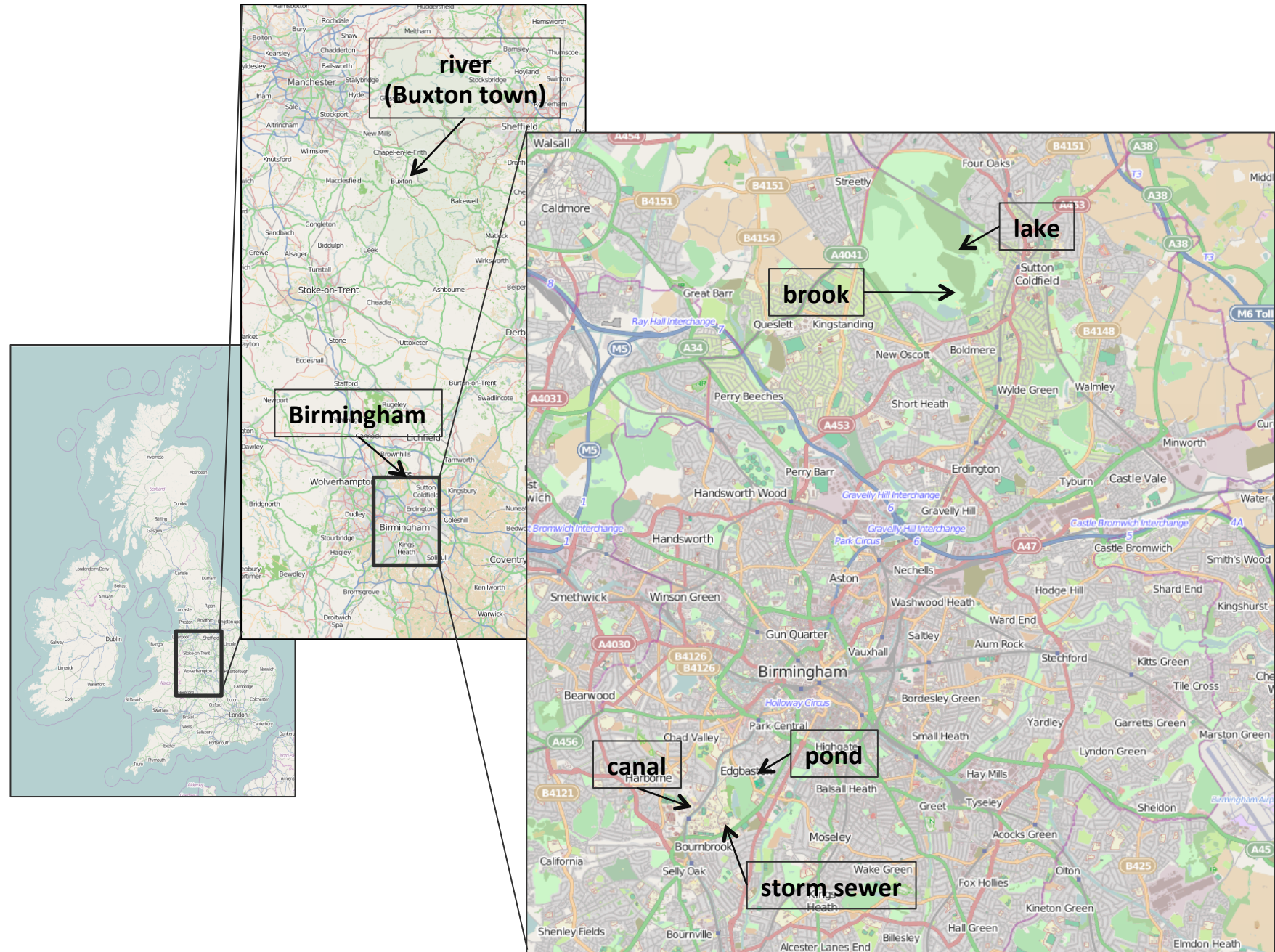
\*SD – standard deviation

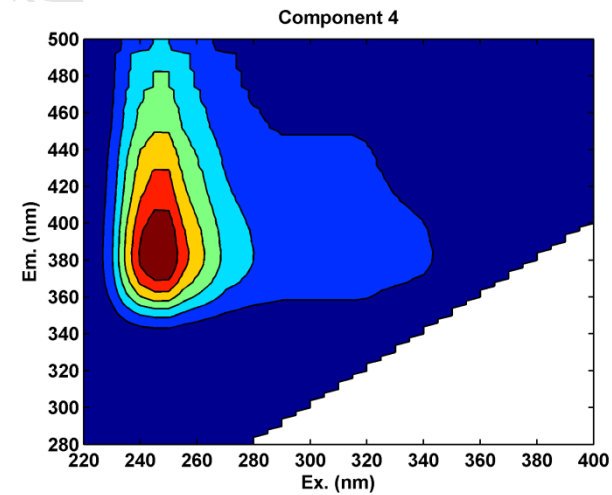
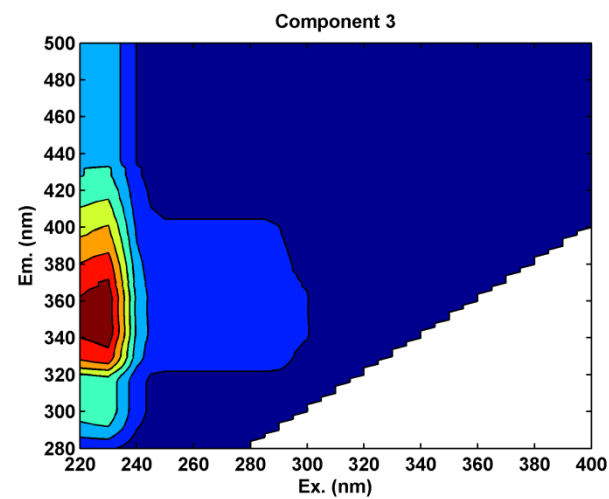
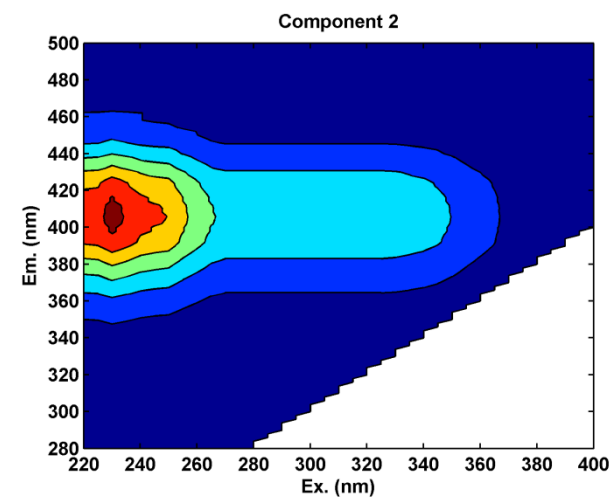
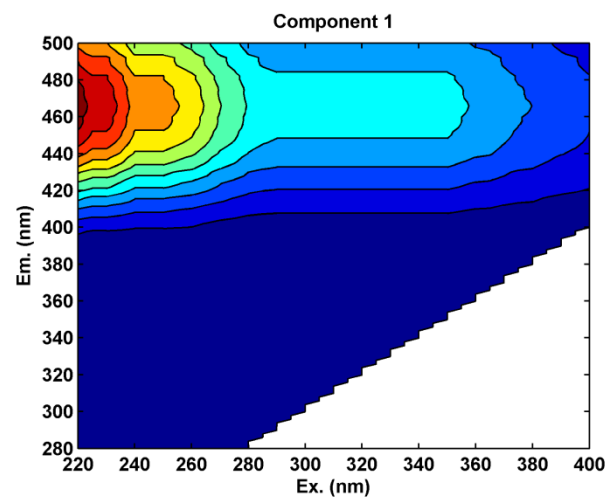
\*\*Calculated according to Yamashita and Jaffe (2008) as percentage of the total fluorescence.

Table 3. Standard data for the analysed water samples.

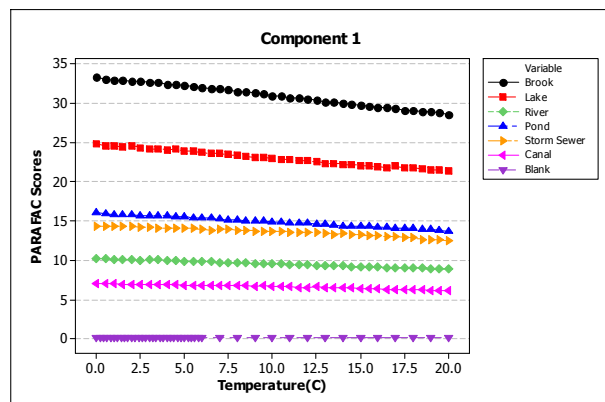
Samples	DOC (mg/L)	pH	Conductivity ( $\mu\text{S}/\text{cm}$ )	Absorbance at 350 nm ( $\text{cm}^{-1}$ )
Brook	7.75	8.1	413	0.089
Lake	8.71	6.8	288	0.078
River	5.55	6.7	340	0.021
Pond	2.96	7.3	687	0.023
Storm Sewer	4.96	7.0	98	0.035
Canal	4.79	6.8	747	0.039



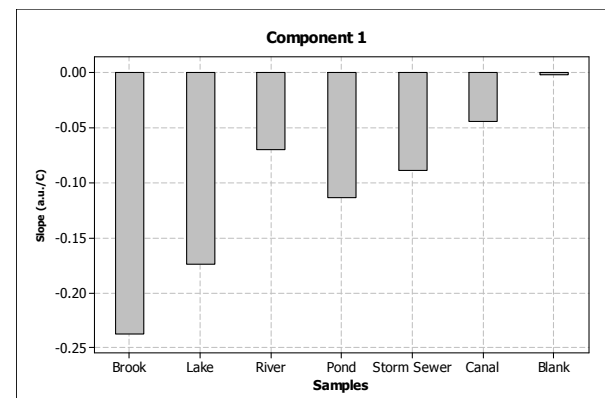




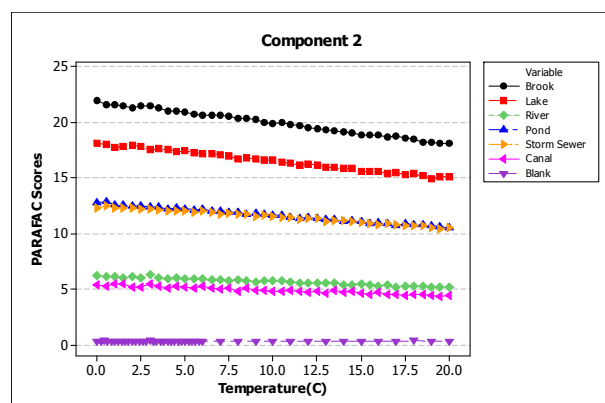




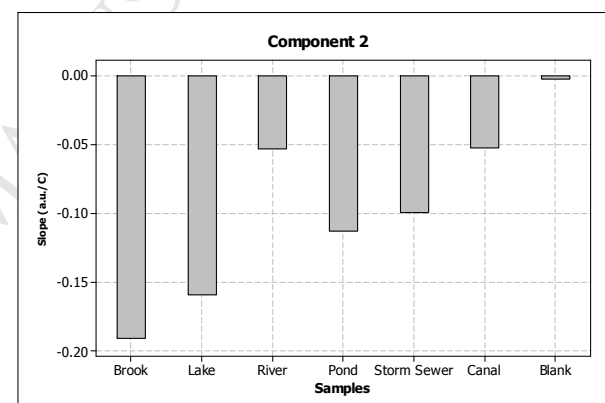
(a)



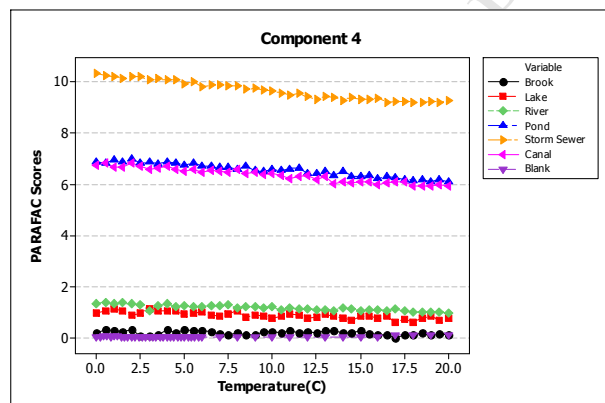
(b)



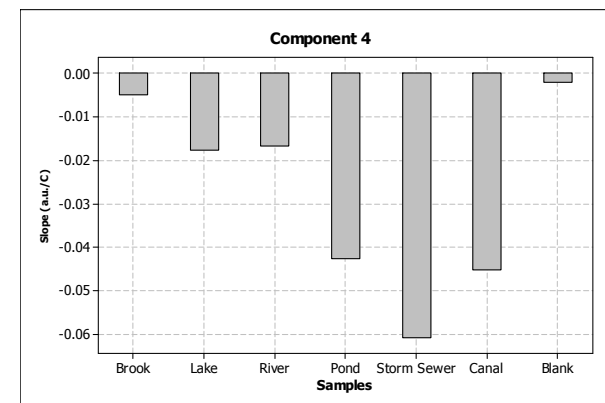
(c)



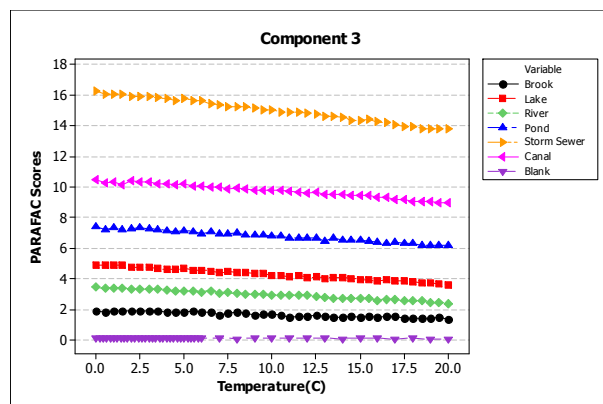
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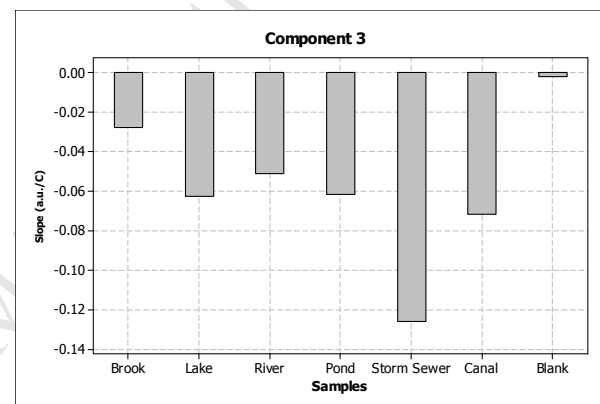
(e)



(f)



(a)



(b)

- We investigated DOM fluorescence properties, at low temperatures, with EEM-PARAFAC
- Fluorescence intensity increases linearly as temperature decreases from 20<sup>0</sup> C to 0<sup>0</sup> C
- DOM PARAFAC components are variably quenched and this is sample specific
- Each humic-like PARAFAC component might be comprised of more than one fluorophore